Q fever - Prevalence and serologic diagnostics in Denmark

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Q fever

Prevalence and serologic diagnostics in Denmark.

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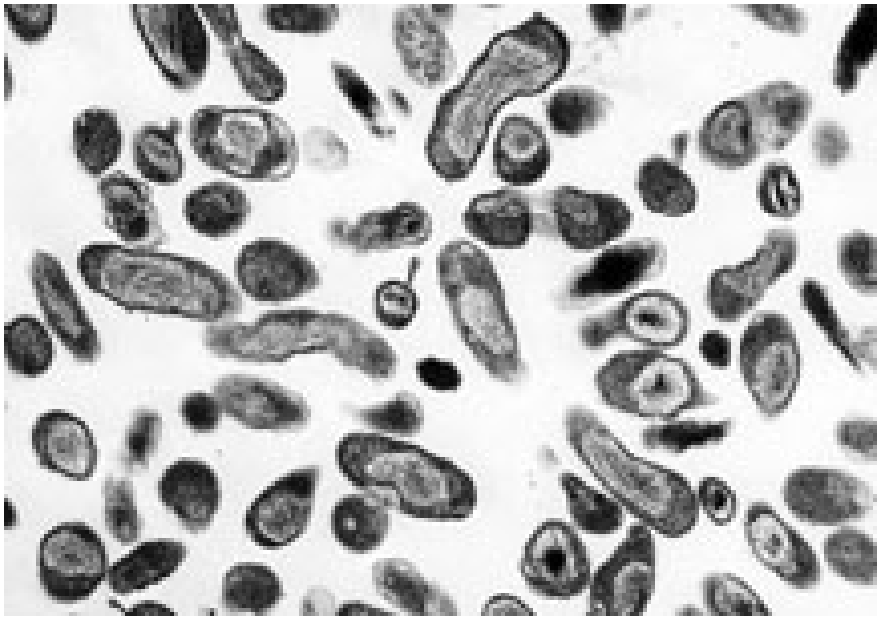


•**Organism Responsible for Q fever**
Rocky Mountain Laboratories, NIAID, NIH

Anna-Bodil Christoffersen
DVM
DTU National Veterinary Institute
Denmark

Q fever occupational disease

- Farmers and their families
- Abattoir workers
- Veterinarians
- Laboratory staff
- Pregnant people

Q fever reservoirs

Tabel 1. Serologic tests for *Coxiella burnetii* from 1989-2004

Blood samples submitted for The Veterinary Institute in connection with export.

Q fever	Cattle		Sheep and goat	
	Tested by CFT	Positive	Tested by CFT	Positive
1989	684	2	-	-
1990	595	6	-	-
1991	102	0	-	-
1992	53	2	76	0
1993	14	0	10	0
1994	175	4	65	0
1995	348	4	-	-
1996	735	0	-	-
1997	814	11	-	-
1998	473	6	-	-
1999	837	30	-	-
2000	396	7	-	-
2001	6	0	-	-
2002	337	0	-	-
2003	225	8	-	-
2004	105	7	-	-

Test methods used in Denmark

- **Serologic** tests: ELISA (IDEXX and LSI kits), CFT, IFAT
- CFT: only for dog, pig, - none ruminants
- IFAT: human samples

- **FISH :**placenta, fetus, bone marrow (Tim Jensen et al. developed and published 2007)

- **PCR** (blod, milk, tissue)
- qPCR and MLVA-PCR (MultiLocusVariableTandemRepeat Assay)

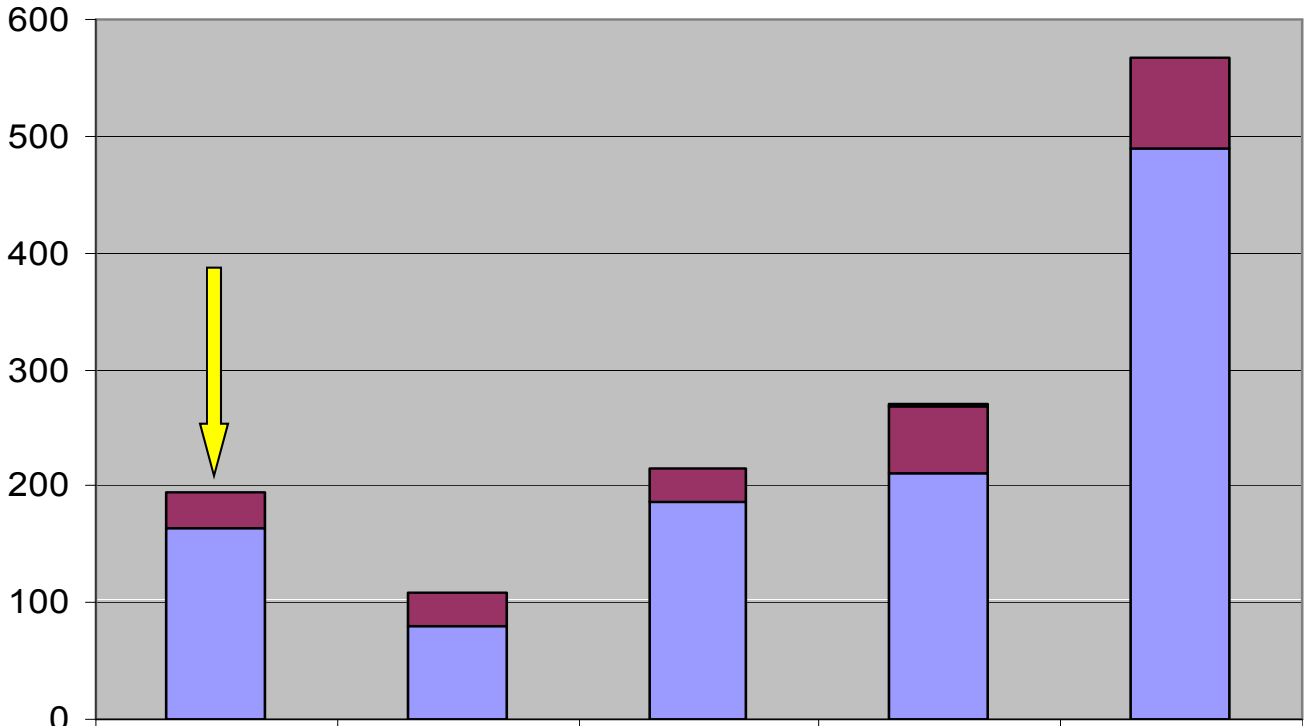
- **Cultivation** in cell cultures and animals (level 3 lab.), not in Denmark

Why did we start looking for antibodies for Q fever in cattle in Denmark?

- A Club- 5 meeting in 2003: AFSSA, VLA, SVA, DVI, CIDC

- Until then only animals for export were tested

Q fever prevalens in blood samples from cattle 2003-2007



	2003	2004	2005	2006	2007
Positive %	18,0%	35,0%	15,0%	27,5%	16,0%
Positive No.	30	28	28	58	78
Total incl. Positive	164	80	187	211	489

Other serologic tests in 2003-2004

- All samples were tested for antibodies for: Leptospirosis, Neosporosis, BVD and Brucellosis
- Exept the Neosporosis: *all other tests were negative*

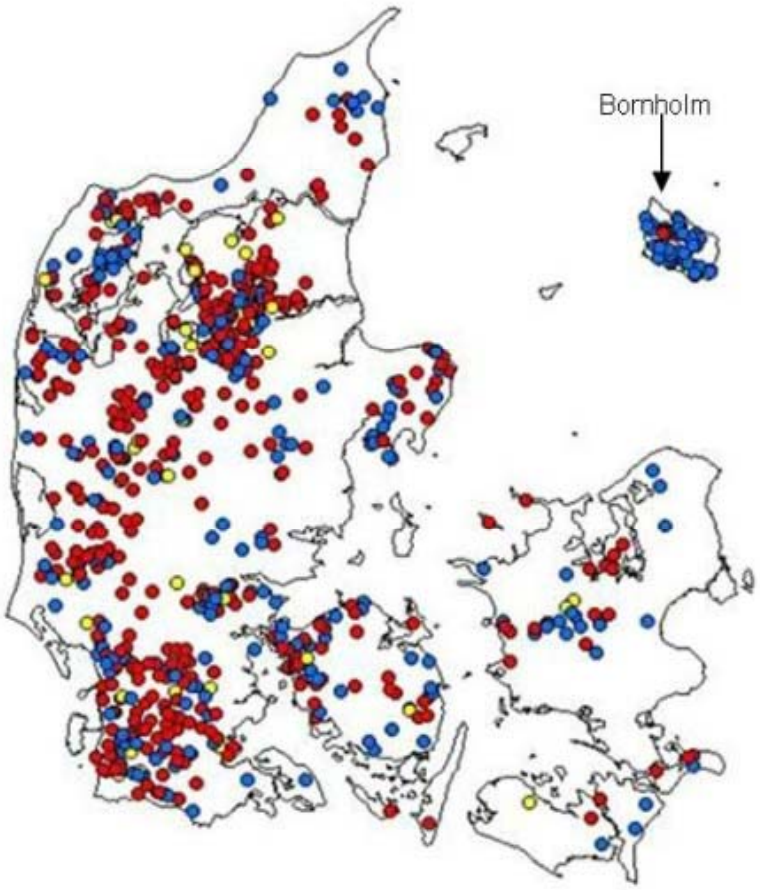
- In 2003: 18 % positive for Q fever and *halv of them were positive for Neosporosis too*
- In 2004: 35 % positive for Q fever, 30% was positive for Neosporosis , *18 % was positive for both Q fever and Neosporosis*

RESULTS OF DETECTION ANALYSIS FOR *Coxiella burnetii* in FOUR SAMPLES FROM DENMARK, AFSSA 2005-06-10

	1.extraction	1.extraction	2.extraction	2.extraction
Samples	PCR1	PCR2	PCR1	PCR2
12643-1	-	- / +	-	-
12249-2	-	-	-	-
12555-3	-	-	-	-
12129-5 ALSO STAMP POS	+	+	+	+
Negative control	-	-	-	-
Positive control	+	+	+	+

Prevalence of Q fever in Denmark 2007

tested in bulk tank milk samples



- 742 farms
- 429 (57%) positive / red
- 276 negative/ blue
- 48 dubious / yellow

Using spatial scan statistic we identified a strong geografical trend with relatively more positive farms in the western part of the country (milk samples 2007). (René Bødker & Anna-Bodil Christoffersen, DVT no. 14. 2008)

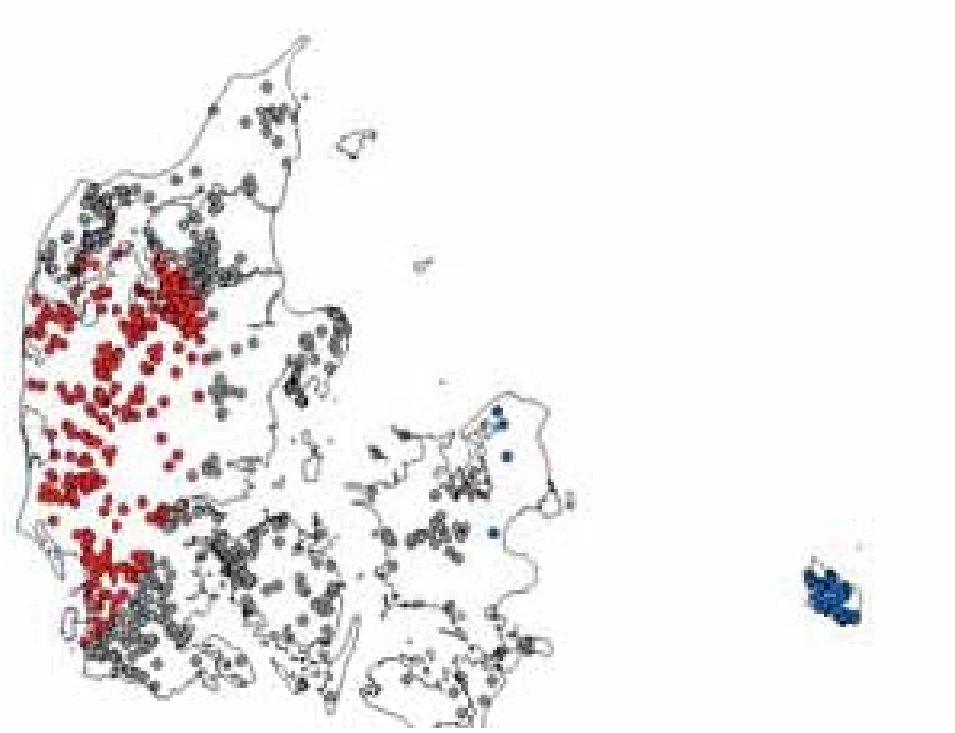
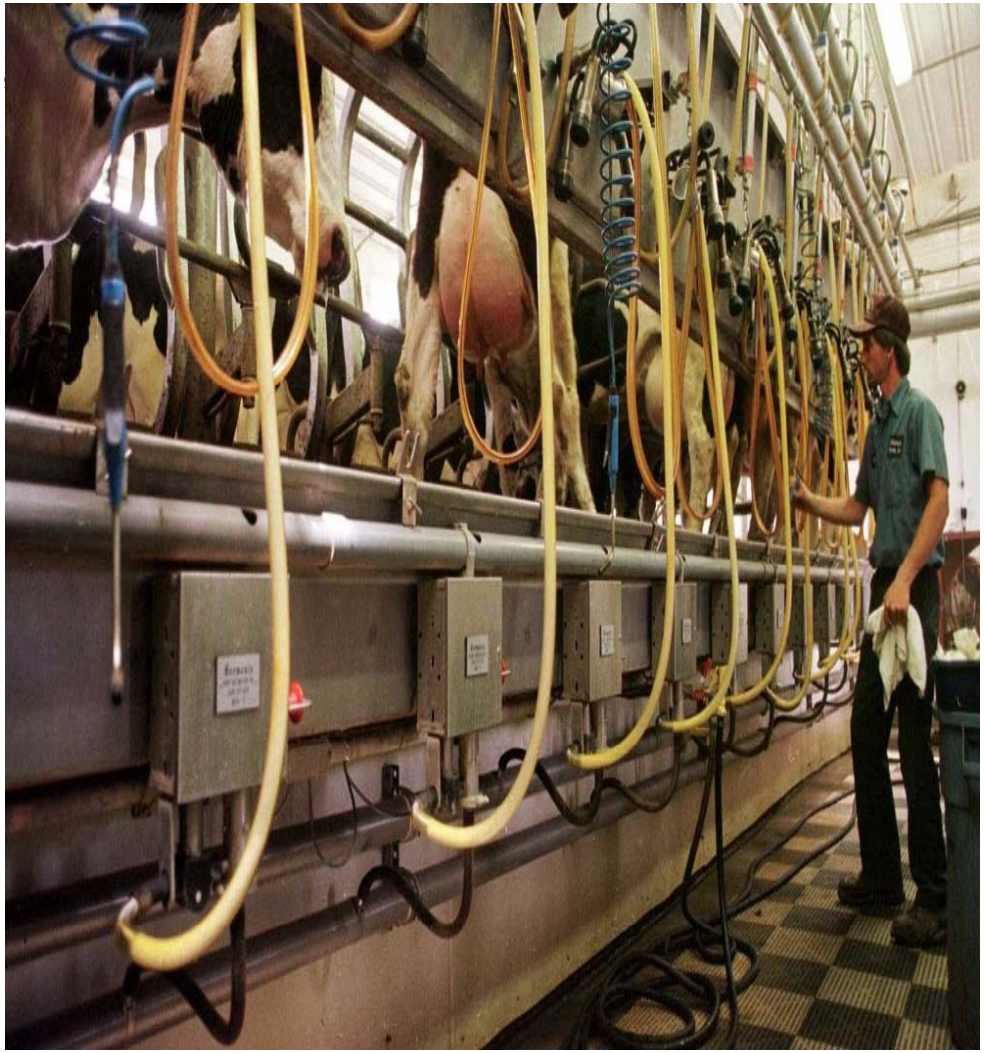


Table 1. **Samples tested for Q fever *(Coxiella burnetii*) by serology or FISH in 2009 at the DTU Veterinary Institute. N = numbers, (pos) = positive samples, C =cases/submissions. Prevalence in bulk milk, cattle: 78,9 %, in individual milk, cattle: 43,5 %, in serum, cattle: 19,8 %. Prevalence by FISH test: 7 %.None positive samples from goat/sheep, other animals.**

	CATTLE	CATTLE	CATTLE	CATTLE	SHEEP/ GOAT	SHEEP/ GOAT	SHEEP/ GOAT	PIG	DOG
	serum	bulkmilk	individual milk	placenta or fetus	serum	milk	placenta	serum	serum
	N(pos)C	N(pos)C	N(pos)C	N(pos)C	N(pos)C	N(pos)C	N(pos)C	N(pos)C	N(pos)C
Purpose									
EXPORT	52 (0) 33	-	-	-	27 (0) 4	-	-	-	-
Diagnostic	111 (22) 69	157 (124) 155	23 (10) 15	14 (1) 14	16 (0) 1	-	-	-	-
Breeding stock	5 (0) 2	-	-	-	2 (0) 1	-	-	-	-
Other countries	-	-	-	.-	-	-	-	-	-
Import	-	-	-	-	16 (0) 2	-	-	-	-
Method									
ELISA	168 (22) 104	157 (124) 155	23 (10) 15	-	61 (0) 8	-	-	-	-
CFT	-	-	-	-	-	-	-	-	-
FISH	-	-	-	14 (1) 14	-	-	-		-

The study of Q fever in Danish dairy cattle herds 2008-2009 (KU-LIFE, Danish Cattle Federation, DTU Vet. Inst.)

- 100 dairy farms were selected randomly and bulk tank milk samples were tested by ELISA
- The farmers were interviewed by telefon (48 questions) and asked to be tested for antibodies against Q-fever, them selves and the staff



The study of Q fever in Danish dairy cattle herds 2008-2009 KU-LIFE, Danish Cattle Federation, DTU Vet. Inst.)

- 23 farms selected from the 100 random selected farms
- Bulk tank milk tested every month (13 times)
- Individual milk samples tested by ELISA and qPCR 3 times from every milking cow
- Blod samples from selected young and adult cattle: testet 3 times in pos farms
- Placenta and abortions investigated in 2 months

Frequency in humans

- **Denmark**:
- Until 2005: few cases per year
- In 2006: 47 seropositive humans
- In 2007: 600 seropositive humans

- ***Data from projects in 2008***: 359 humans tested
  - 37% of 87 vets. were positive
  - 2% of 15 insem.were positive
  - 3 % of 163 farmers were positive
  - 7 % of 14 hoofcutters were positive

Preliminairy results (KU-LIFE, Danish Cattle Federation, DTU Vet. Inst.)

- 2.294 blodsamples, 6.828 individual milk samples and 300 bulk tank milk samples were tested

- In positiv farms:
  - 15-60 % individuals pos in milk serology
  - 10-25 % individuals pos in blod serology
  - 2-95 % individuals pos by qPCR
  - few pos milk samples in negative farms

- Results still on calculation

Preliminary results qPCR

1688 milk samples analysed from 12 dairy herds,
40-96 samples from each herd
Number of PCR-positive samples varies between 2-95%,
C_T-range 20-38

		Runde 1			Runde 2			Runde 3			
		Undersøgt	Positive	PCR%	Undersøgt	Positive	PCR%	Undersøgt	Positive	PCR%	
46008	6	46	14	30	46	32	70	40	38	95	
31252	21				46	4	9	41	9	22	
38946	22	44	13	30	44	7	16	45	1	2	
54645	29				57	28	49	48	15	31	
31530	54	44	16	36	45	19	42	43	19	44	
54198	60	40	32	80	52	13	25	44	9	20	
49701	69	50	19	38	42	12	29	43	11	26	
33350	78	42	7	17	46	9	20	46	2	4	
45976	88	96	43	45	94	30	32	90	27	30	
38531	96	43	41	95	46	4	9	44	2	5	
50246	108	47	4	9	46	40	87	43	37	86	
52508	109	47	7	15	52	3	6	46	1	2	
		499	196	**39**	616	201	**33**	573	171	**30**	1688

Prevalence of *Coxiella burnetii* antibodies in Danish dairy herds

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Abstract

During recent years in Denmark higher rates of antibodies to *Coxiella burnetii* have been detected in animals and humans than previously reported. A study based on bulk tank milk samples from 100 randomly selected dairy herds was performed to estimate the prevalence and geographical distribution of antibody positive dairy herds. Using the CHEKIT Q-Fever Antibody ELISA Test Kit (IDEXX), the study demonstrated a prevalence of 59% antibody positive herds, 11% antibody intermediate herds and 30% antibody negative herds based on the instructions provided by the manufacturer. The geographical distribution does not indicate a relationship between the regional density of dairy farms and the prevalence of antibody positive dairy farms. The result supports the hypothesis of an increase in the prevalence of positive dairy herds compared to previous years.

- **_Coxiella burnetii_ associated placental lesions and infection level in parturient cows**
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- **Abstract**
- Several studies have shown vaginal excretion of *Coxiella burnetii* in cattle. However, information on the interaction between the placenta and the bacterium is very limited. To evaluate this, cotyledonary specimens (n = 170) from 19 dairy herds were analysed for *C. burnetii* by real-time PCR targeting the *IS1111a* and *icd* genes. Positive cases (n = 90) and a random selection of negative cases (n = 20) were examined by histology, immunohistochemistry (IHC) and if the infection level was high, also by fluorescence in situ hybridization (FISH). PCR results were compared to bulk tank milk (BMT) antibody levels around the sampling period. Placental infection as determined by rt PCR was found in cows irrespectively of the herd BMT antibody level, but the frequency of cows having placental infection was generally higher in herds with intermediate or high BTM antibody level than in herds with low antibody level. Histological examination revealed a range of mostly mild cotyledonary changes. It was impossible by blinded examination to identify infected cases, but statistical analyses showed associations of unknown significance. One case had *C. burnetii* antigens in multiple trophoblasts, but remained negative by FISH. The presence of antigens was not associated with inflammation. The study indicates that *C. burnetii* infection in the placenta of parturient cows is rarely associated with inflammation. The lack of lesions may explain why bovine Q fever mostly remains clinically unnoticed. Furthermore, the study confirms that cattle originating from herds with negative BTM antibody levels may shed *C. burnetii* at calving.

EPI-NYT
STATENS SERUM INSTITUT · OVERVÅGNING OG FOREBYGGELSE AF SMITSOMME SYGDOMME
Sundhedsstyrelsens Meldesystem for Smitsomme Sygdomme

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Q-FEBER: PRØVETAGNING OG RISIKOHÅNDTERING

Uge 51, 2007

Q-feber er en bakteriel zoonose forårsaget af Coxiella burnetii, EPI-NYT 46/06. Indtil 2005 antog man, at C. burnetii var sjælden i Danmark. Undersøgelser af prøver fra kvægbesætninger udført af Veterinærinstituttet, DTU, har imidlertid vist en høj forekomst af C. burnetii. Der blev påvist en seroprævalens i enkeltdyr på 17% og en seroprævalens i besætninger på > 50%; dette er tegn på aktuel eller tidligere infektion, www.food.dtu.dk.

Forekomst hos mennesker

Q-feber hos mennesker er ikke anmeldelsespligtig, men diagnostik foretages kun på SSI. Indtil 2006 påvistes kun sporadiske, importerede tilfælde. Fra 2006 til november 2007 er undersøgt omkring 650 personer for antistoffer mod C. burnetii. Hos 1 % af personerne kunne påvises forhøjet antistof som tegn på tidligere eller aktuel infektion. Størstedelen af de undersøgte personer havde tilknytning til kvægbrug. De fleste havde ikke symptomer på Q-feber, men havde muligvis været udsat for smitte fra positive besætninger. Endvidere er der fundet flere tilfælde med serologiske tegn på aktuel Q-feber hos gravide kvinder med erhvervsmæssig kontakt til kvægbesætninger.

Risikogrupper

De fleste tilfælde er Q-feber forårsaget af persisterende feber, EPI-NYT 46/06. Der kan være symptomer, som også ses ved atypisk pneumoni.

- Gravide, der har været i kontakt med besætninger, hvor der er øget

Risikohåndtering i dyrebesætninger

Dyr med Q-feber udskiller bakterier i højt antal i moderkagen. Der er risiko for smitte via aerosoler, især i forbindelse med fødsler, aborter eller kontakt med efterbyrder. Arbejdstilsynet og Sundhedsstyrelsen anbefaler, at gravide og personer med svækket immunsystem og/eller kroniske hjertesygdomme, især i form af hjerteklapsygdomme, ikke opholder sig i kvæg-, fåre- eller gedebesætninger med abortproblemer, hvor en infektiøs årsag mistænkes. Hvis ærindet er tvingende, anvendes åndedrætsværn med P3 filter.
Det er desuden vigtigt at skaffe fødselsmateriale af vejen på forsvarlig vis i Q-feber positive besætninger, www.lr.dk.

www.lr.dk.

Kommentar

Tabel 1. Behandlingsudfald for alle patienter med tuberkulose, uanset lokalisation, 2005

Behandlings-fald	Danskere	%	Indvandrere	%
Helbredt	55	33	58	23
Gennemført	87	52	163	64
Beh.succes	142	86	221	86
Død	14	8	5	2
Mistet	1	1	0	0
Udredt	3	2	12	5
Overført	3	2	10	4
Ukendt/ukendt	3	2	8	3
	166	100	256	100

Tabel 2. Behandlingsudfald for alle patienter med dyrkningspositiv lungetuberkulose, 2005

Behandlings-fald	Danskere	%	Indvandrere	%
Helbredt	50	36	49	26
Gennemført	66	48	110	58
Beh.succes	116	85	159	84
Død	13	9	4	2
Mistet	1	1	0	0
Udredt	3	2	10	5
Overført	2	1	10	5
Ukendt/ukendt	2	1	6	3
	137	100	189	100

De mulige udfald er: 1. Helbredt, 2. Gennemført behandling, 3. Død,

What can we do to get in front of the disease?

- **Educate the public on source** af infektion (infection by inhalation still the main route ?)

- Source of infektion in calves,lambs,kids: **inhalation or neonatal infektion, calving, placenta, amniotic fluid, raw milk ?**

- Positive farms / pets: **Appropriate removal of placenta/ aborted material - How?**

- Abattoir workers: **careful remove and destroy mammary glands**, inner organs, keep skin wett until salting (Aitken et al 1987)

- **Use only pasteurized milk and milk products-** Why do we still eat cheese produced from ”lait cru”? What about meat?

- **Vaccination: Calves,kids,lambs, special human groups (vets,others)?**

- **Handling manure – how?**

Can we do anything intelligent to get in front of this disease?

- FACTS:

- Notifiable disease among animals since 2005 in Denmark
- No surveillance program at the moment
- Voluntary interest from vets. and cattle farmers: > 60 % pos cattle farms
- Goat/sheep: les than 100 blood samples tested pr year, few bulk milk samples, untill now none pos

- What about:
- Instructions for visitors in pos farms?
- Visitors in ZOO ?
- Instructions for owners of pets (cats, dogs, domestic and wild birds)?
- Manure?

Prevalence in dogs and cats.

German investigation (Werth 1987)

1127 dogs : 13 % seropositive

108 cats : 26% seropositive

Prevalence in Denmark unknown

Outbreake of Q fever in the Netherlands

The Netherlands: 2-20 human cases pr. year until 2006

In 2008: 677 cases, one outbreake

In 2009: > 2000 cases in North Brabrand

Dairy goat and sheep main source

One special subtype of *C. burnetii* in nearly all dutch cases (MLVA PCR technique)

> 600 dairy goat farms in The Netherlands
several farms have more than 1000 goats

Vaccination of goat and sheep in the Netherlands 2009-2010

CEVA: phase 1 vaccine (Coxevac$^{R)}$

- *C.burnetii* free can be vaccinated
  - lower excretion in milk and placenta
  - prevent most abortions (goats /sheep)

>60.000 not vaccinated pregnant goat/sheep killed in the Nederlands 2009/2010

Sufficient vaccine in The Netherlands for all goats and sheep in 2010

Per oral dosis of *C. burnetii*

Experimental in mice (Durand, 1993)

Mice needs 10.000 times more of *C. burnetii* by per oral route than by the intraperitonal route.

Methods: cultivation in eggs and serology by CFT

Q Fever vaccine for humans (Stephen Graves, Australia)

•Australia is the only country with an approved human vaccine against Q fever
•Developed by Marmion and Ormsbee

•Produced by Australian company “CSL Biotherapies”in Melbourne

•Licensed for use in 1989

•A formalin-killed phase 1 whole cell **Henzerling strain** grown in the embryonated egg yolk sac.

•20 years experience and approximately

• 200.000 doses have been given.

Q Fever Vaccine (Stephen Graves, Australia)

Pre-screening of proposed vaccine recipients necessary to prevent adverse reactions to vaccine

Day 1:
a)Blood taken for *C.burnetii* serology
b)Intradermal skin test of cell-mediated immunity to *C.burnetii*(a “Mantoux”–like test)
c)Take medical history of Q Fever

Day 7:
a)No antibodies to *C.burnetii*
b)No T-cell reaction (no induration) to *C.burnetii*
c)No history of Q Fever

THEN
d)Vaccinate with 25µg vaccine (0.5ml) (subcutaneous) x1 dose only.

Day 21:
a)Patient now immune to Q Fever
b)No need to check for seroconversion

Duration of vaccine protection uncertain but likely to be approx. 10 years


Thank you for listening